

Daffodil Chromoplast DNA: Comparison with Chloroplast DNA, Physical Map, and Gene Localization

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Z. Naturforsch. **42c**, 118–122 (1987); received August 14, 1986

Narcissus, Chromoplast DNA, Chloroplast DNA, Physical Map, Gene Localization

In daffodil, development of chloroplasts or chromoplasts is not accompanied by plastid DNA modification. This has been shown by comparing the fragment pattern of different restriction endonucleases, and by the lack of methylation of CCGG sequences. A physical map has been constructed for the plastome using the restriction endonucleases *Sal* I, *Pst* I, and *Bgl* I. The fragments containing the genes for the large subunit of ribulose biphosphate carboxylase/oxygenase (*rbcL*), the *alpha*, *beta*, and *epsilon* subunits of the ATP synthase complex (*atpA*, *atpB*, *atpE*), cytochrome *f* (*petA*), and for the 51 kDa chlorophyll apoprotein of photosystem II (*psbB*) have been identified. The respective gene locations correspond to those on spinach chloroplast DNA.

Introduction

According to the endosymbiont hypothesis, chloroplasts phylogenetically represent the original plastid type [1]. In the process of evolution, other plastid types with different functions have evolved in connection with the development of organs and corresponding cell differentiation. During ontogeny, the various types of plastids within a plant all develop directly or indirectly from proplastids, and they are interconvertible [2]. Therefore one might suppose that all plastidal forms of a single plant contain either the same, or a reversibly modified, genetic material. Modification through methylation of ptDNA has been found in the amyloplasts of sycamore [3] and during gametogenesis in *Chlamydomonas* [4].

Preliminary investigations revealed no apparent differences between the DNA of daffodil chromoplasts (crDNA) and chloroplasts (clDNA) [5], although these plastid types differ greatly in pigment content, fine structure, and lipid as well as protein composition [6]. The protein complements of the two plastid types, in particular, are very different [7]. The plastome of the daffodil appears to become inactivated during development of chromoplasts from prochromoplasts [8]. The aim of the present work was to ascertain whether DNA modifications occur in parallel to the changes found in the structure and

protein composition of nucleoids during chloro- and chromoplast differentiation [9]. Further, in continuation of earlier work on the daffodil plastome [5, 9–14] and as a basis for further comparative studies on the genetic material of chloro- and chromoplasts, a physical map of the plastome together with the localization of some genes is presented.

Material and Methods

Daffodils (*Narcissus pseudonarcissus* L. cv. 'Golden Harvest') were bought from the local market. Chromoplasts from the daffodil coronae were isolated as described by Thompson *et al.* [14]. For isolation of the chloroplasts, 120 g of green leaves were homogenized in 600 ml buffer A (0.33 M sorbitol, 50 mM Tris/HCl pH 8.0, 3 mM Na₂EDTA, 1 mM 2-mercaptoethanol, 0.1% (w/v) BSA) in a blender (Bühler, FRG). After filtration through three layers of nylon tissue and low speed centrifugation (5 min, 130 × g), the chloroplasts were pelleted from the supernatant (60 s, 4000 × g), resuspended in buffer A, and repelleted (60 s, 4000 × g). The pellet was resuspended in buffer A and overlaid onto discontinuous Percoll density gradients (0, 36, 72% (v/v) Percoll, each in buffer B (0.3 M sucrose, 50 mM Tris/HCl pH 8.0, 3 mM Na₂EDTA)). After centrifugation in a swinging bucket rotor (8 min, 4000 × g), the lower green band (36/72% Percoll) containing the intact chloroplasts was removed, and diluted 1:3 with buffer A. After a final centrifugation (2 min, 4000 × g), the chloroplasts were stored at –70 °C as pellets prior to use. From plastidal pellets the

Abbreviations: BSA, Bovine Serum Albumine; clDNA, Chloroplast DNA; crDNA, Chromoplast DNA; ptDNA, Plastid DNA.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0100–0118 \$ 01.30/0



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ptDNA was extracted according to Kolodner and Tewari [15], and concentrated and stored as described by Herrmann [16].

Primary digestions of the DNA by means of restriction endonucleases were carried out under conditions recommended by the suppliers. Double digestions were carried out consecutively, adjusting the ionic conditions for the second enzyme. For construction of the physical map the crDNA and the restriction enzymes *Sal* I, *Pst* I, and *Bgl* I were used. The orientation of the fragments was determined as described by Herrmann *et al.* [17]. Several genes were localized on the physical map of the chromoplast DNA using genespecific heterologous cloned spinach cDNA fragments (*cf.* Table I). The following procedures were applied: Southern blot [18], nick translation [19], hybridization [20].

Results and Discussion

Recent investigations have shown that the ptDNA molecules within one plant need not be identical. Microheterogeneity (*Euglena gracilis* [21]), inversions of whole regions within the plastome [22], and even different DNA molecules (*Acetabularia acetabulum* [23]; brown algae [24]) have been reported as variants of a seemingly uniform plastome. This data, plus the finding that some methylation occurs in amyloplast DNA of sycamore (*Acer pseudoplatanus*) suspension culture cells [3], may be taken as an indication that, during the development of the various plastid types, the plastome may be modified in some way.

During tomato fruit ripening the expression of several genes declines, and, with the exception of the mRNA for the 32 kDa protein, the corresponding mRNAs are no longer detectable in the chromoplasts of ripe fruits [25]. In the daffodil the plastome appears to become totally inactivated during chromo-

plast development [8]. Iwatsuki *et al.* [26] and Thompson [5] have shown already that cr- and cDNA are probably identical. This finding is confirmed in the present paper for the plastome of the daffodil. The ptDNA from chromoplasts and chloroplasts yield identical fragment patterns on digestion with six different restriction endonucleases (Fig. 1). Furthermore, endonuclease digestion with *Hpa* II, which only cuts the unmethylated sequence C/CGG, shows that there are no differences in the methylation of this sequence (Fig. 1f). The fragment pattern of crDNA after *Msp* I digestion – this enzyme cuts the sequence C/GGG as well as the methylated sequence C/CmGG – is identical to the pattern yielded by *Hpa* II digestion (Fig. 1g). This means that neither of the ptDNAs are methylated in the sequence CCGG, *i.e.*, cr- and cDNA appear to be identical. The possibility that gene expression is regulated by methylation, as in eukaryote nuclear DNA, can thus be ruled out in the case of daffodil chromoplasts. The regulation of gene expression during chloroplast and

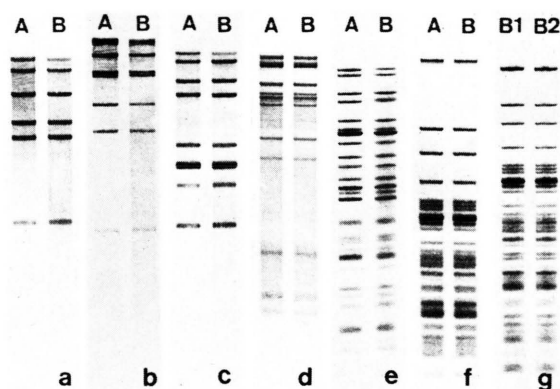


Fig. 1. Comparison of chloroplast (lane A) and chromoplast (lane B) DNA from the daffodil after *Pst* I (a), *Sal* I (b), *Bgl* I (c), *Sac* I (d), *Bam* HI (e), *Hpa* II (f), *Msp* I (g; B1) and *Hpa* II (g; B2) endonuclease treatment.

Table I. Chloroplast DNA clones from spinach used as probes for heterologous hybridizations (for nomenclature *cf.* [33]).

Genes	Primary clone/subclone	Fragments used	Probe size (bp)
atpA	pWHsp 210	Taq I-1 + 2	700 and 600
atpB	pWHsp 403/E 1	Sac I-1	1200
atpE	pWHsp 403/E 3	Xba I-2	420
rbcL	pWHsp 403/E 2	Pst I-1	1260
petA	pWHsp 407/E 4	E 4 total	830
psbB	pWHsp 207/E 3	E 3 total	1450

chromoplast development therefore does not appear to be due to changes at the DNA level. Epigenetic factors such as structure, composition and protein complement of the nucleoids seem to play a predominant role in differential gene expression of plastids [9].

Earlier investigations have shown the daffodil plastome DNA to be circular with a length of 161 kbp. Furthermore, the 16S and 23S rRNA genes were shown to be localized in an inverted repeat sequence [12, 14]. A physical map was constructed using the restriction endonucleases *Pst* I, *Sal* I, and *Bgl* I (for fragment patterns, cf. Fig. 1 a–c). The respective fragment sizes could be ascertained on calibrated gels from marker DNAs (Table II). By means of consecutive digestion with different enzymes, a physical map for the daffodil crDNA for the three enzymes applied could be constructed (Fig. 2). The locations of the *Sal* I fragments S3 and S7 were determined with the help of a fourth enzyme (*Sac* I). Circularity and overall size of the daffodil genome, as determined by Thompson *et al.* [14], could be confirmed by the physical map as presented here.

Comparative analyses of the organization of ptDNAs from a variety of higher plants revealed a very stable order of genes. The spinach gene order and arrangement appears to be the ancestral one. In some cases, however, the gene arrangement is changed by inversions (cf. [27]). In order to locate the genes for certain proteins in the daffodil plastome, the ptDNA was digested with *Sal* I, *Sal* I + *Pst* I, *Bgl* I, and *Bgl* I + *Pst* I, and the resulting fragments were separated in agarose gels. Fig. 3 shows ethidium bromide-fluorescence photographs of the gels, which were used for Southern transfer, together with their corresponding autoradiograms for each localized gene. The position of the genes is shown in

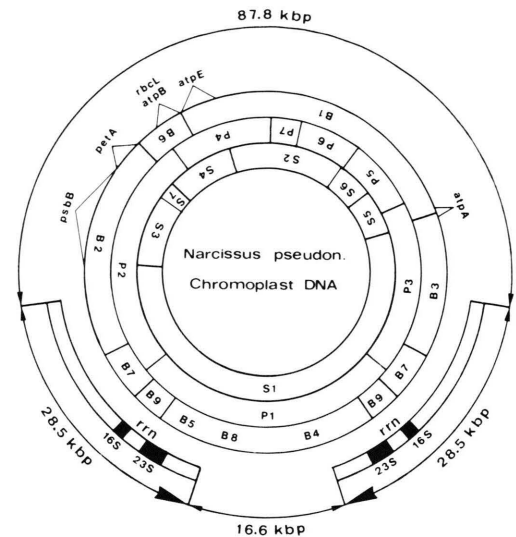


Fig. 2. Physical map of the daffodil plastome. Inner part: Map of the restriction endonuclease sites (S = *Sal* I; P = *Pst* I; B = *Bgl* I); the positions of 6 genes are indicated. Outer part: Organization of the plastome in a large single copy region (87.8 kbp), inverted repeat (28.5 kbp), and a small single copy region (16.6 kbp); the positions of the 16S and 23S rRNA genes are indicated (cf. [14]).

Fig. 2. The genes appear to be located in approximately the same position within the plastome of the daffodil (*Liliales*) as in the case for spinach. Investigations on the hybridization kinetics of heterologous ptDNAs have shown that the homology value between monocotyledons and dicotyledons is below 30% (daffodil/pea: 27%; [28–30]). Such a low degree of sequence homology is surprising in view of the highly conserved gene organization of the plastome, and the more so as the majority of the ptDNA is expected to consist of coding regions [31, 32].

Table II. Restriction fragment sizes of chromoplast DNA from the daffodil.

<i>Sal</i> I	Size (kbp)	<i>Pst</i> I	Size (kbp)	<i>Bgl</i> I	Size (kbp)
S1	90.8	P1	45.9	B1	43.9
S2	25.6	P2	41.7	B2	33.0
S3	15.2	P3	29.7	B3	21.3
S4	13.9	P4	17.6	B4	16.9
S5	9.1	P5	12.1	B5	8.9
S6	7.2	P6	10.3	B6	7.5
S7	3.2	P7	5.0	B7 (2×)	7.4
				B8	6.5
				B9 (2×)	4.8

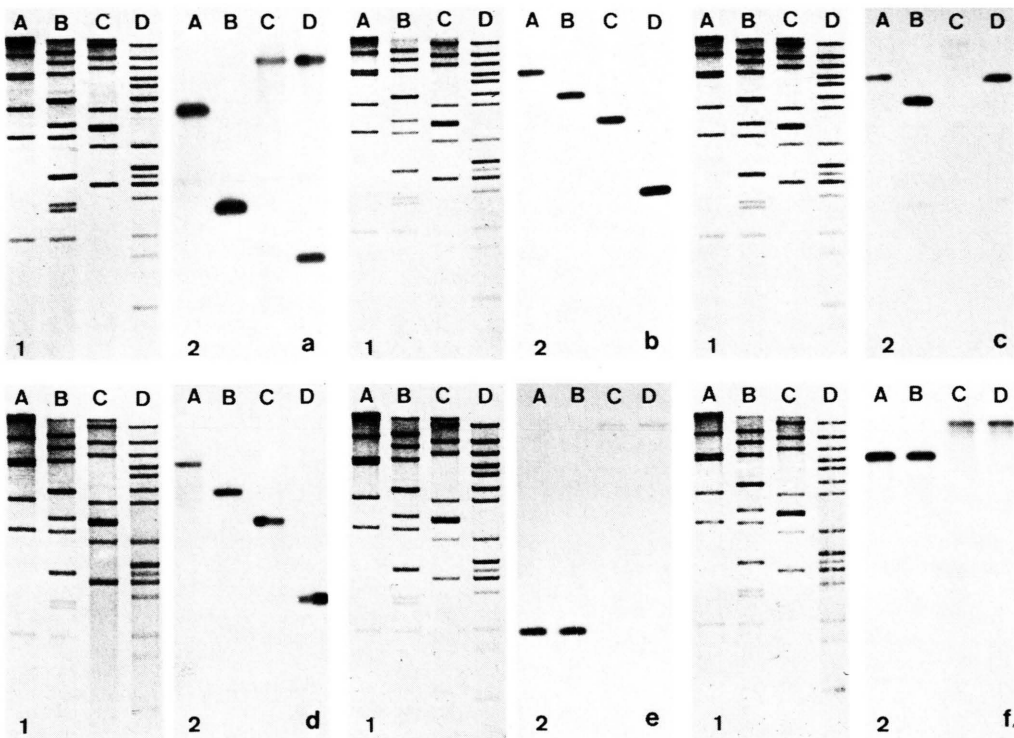


Fig. 3. Localization of the genes for the *alpha*- (atpA; a), *beta*- (atpB; b), *epsilon*- (atpE; c) subunits of the ATP synthase complex, large subunit of the ribulose-bisphosphate carboxylase (rbcL; d), cytochrome *f* (petA; e), and 51 kDa chlorophyll apoprotein of the photosystem II (psbB; f). Daffodil chloroplast DNA digests (lane A: *Sal* I; B: *Sal* I + *Pst* I; C: *Bgl* I; D: *Bgl* I + *Pst* I) were separated in 0.6% agarose gels, filter immobilized and hybridized with cloned spinach chloroplast DNA fragments containing only structural parts of the genes (cf. Table I). 1) Ethidium bromide fluorescence photographs; 2) autoradiograms.

Acknowledgements

This study was supported by the Deutsche Forschungsgemeinschaft (grant to Prof. P. Sitte). I thank Prof. R. G. Herrmann for providing me with

the gene probes, Dr. C. Bisanz-Seyer for carrying out the hybridizations, Prof. P. Sitte for helpful discussions, and Dr. C. Beggs for checking the English text.

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